

FORM PTO-1390
(REV 12-29-99)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

5367-US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/582952INTERNATIONAL APPLICATION NO.
PCT/US99/00475INTERNATIONAL FILING DATE
09 January 1999PRIORITY DATE CLAIMED
09 January 1998


TITLE OF INVENTION
SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF

APPLICANT(S) FOR DO/EO/US

G.R. PETTIT et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
 2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
 3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
 4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
 5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
 6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
 7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
 8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
 9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
 10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11. to 16. below concern document(s) or information included:**
11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
 12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
 13. ☐ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
 14. ☐ A substitute specification.
 15. ☐ A change of power of attorney and/or address letter.
 16. ☒ Other items or information: Copies of Verified Statement Claiming Small Entity Status - Independent Inventor as originally filed with US Provisional 60/070,878 filed January 9, 1998
Certificate of Express Mail

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/582952		INTERNATIONAL APPLICATION NO. PCT/US99/00475		ATTORNEY'S DOCKET NUMBER 5367-IIS	
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$970.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	5 - 20 =	0	X \$18.00	\$ 0	
Independent claims	5 - 3 =	2	X \$78.00	\$156.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$252.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$126.00	
SUBTOTAL =				\$126.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$ 126.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$	
TOTAL FEES ENCLOSED =				\$ 126.00	
				Amount to be refunded:	\$
				charged:	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>126.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>13-5100</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Richard R. Mybeck 8010 East Morgan Trail, Suite 10 Scottsdale, Arizona 85258-1234 480.483,1285			 SIGNATURE: Richard R. Mybeck NAME 17,886 REGISTRATION NUMBER		

APPLICANT: GEORGE R. PETTIT et al.

DOCKET NO. 5367

SERIAL NO.:

FILED:

FOR: SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF

VERIFIED STATEMENT CLAIMING SMALL ENTITY
STATUS - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF described in the specification filed herewith.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

I acknowledge the duty to file, in this application, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon to which this verified statement is directed.

Date 1/7/95

George R. Pettit
GEORGE R. PETTIT

APPLICANT: GEORGE R. PETTIT et al. DOCKET NO. 5367
SERIAL NO.:
FILED:
FOR: SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF

VERIFIED STATEMENT CLAIMING SMALL ENTITY
STATUS - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF described in the specification filed herewith.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

I acknowledge the duty to file, in this application, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon to which this verified statement is directed.

Date

1/7/97


BRIAN TOKI

Synthesis of Phenstatin and Prodrugs Thereof

5 The present invention relates generally to compounds for use in the treatment of one or more neoplastic diseases and more particularly to the synthesis of the antineoplastic compound herein denominated "phenstatin" and water soluble prodrugs thereof.

This research was funded in part by Outstanding Investigator Grant CA44344-01-09 awarded by the National Cancer Institute, DHHS. The United States government may have certain rights to this invention.

10 By way of background, the elucidation and isolation of combretastatin A-4 is described in U.S. Patent No. 4,996,237 which issued to G.R. Pettit et al., on February 26, 1991, while early efforts to develop a combretastatin A-4 prodrug are described in U.S. Patent No. 5,561,122, which issued to G.R. Pettit on October 1, 1996. The general background information from each of those patents is incorporated herein by this reference thereto.

15 The potent cancer cell growth and tubulin assembly inhibitor combretastatin A-4 was originally isolated from the African tree *Combretum caffrum* (*Combretaceae*) circa 1985 and has been undergoing clinical development. The present disclosure comprises a benchmark in the continuing effort to synthesize practical water soluble prodrugs related to combretastatin A-4 and is a significant and remarkably unexpected extension of those early efforts which are described in U.S. Patent No. 5,561,122, *supra*.

20 The African willow tree *Combretum caffrum* Kuntze (*Combretaceae*) has proven to be a very productive source of cancer cell growth (murine P388 lymphocytic leukemia) inhibitory stilbenes, bibenzyls and phenanthrenes (See: Pettit, et al., Antineoplastic Agents 291. Isolation and Synthesis of Combretastatins A-4, A-5, and A-6, *J. Med. Chem.* 1995, 38, 1666-1672). Since 25 1979 promising leads have been pursued which were focused on the three most active (inhibition of cancer cell growth and polymerization of tubulin (*Id.*))

constituents, namely combretastatin A-1 (**1a**), A-2 (**2**), and A-4 (**1b**) (*Id.*) (See, Figure 1). Of these, combretastatin A-4 (**1b**) has reached clinical development as the very soluble prodrug **1d**. Meanwhile other research groups have also been further extending structure/activity relationships (hereinafter referred to as "SAR") among the combretastatins (See: Bedford, et al., Synthesis of Water-Soluble Prodrugs of the Cytotoxic Agent Combretastatin A-4, *BioMed. Chem. Lett.* 1996, 6, 157-160) and related stilbenes (See: Kitanaka, et al., Oligomeric Stilbenes from *Caragana chamlagu* LAMARK Root, *Chem. Pharm. Bull* 1996, 44, 565-567).

Epoxidation of combretastatin A-4 silyl ether (**1c**) (Pettit et al., 1995, *supra*) using the Jacobsen chiral Mn (salen) complex (See: Popisil, et al., X-Ray Structural Studies of Highly Enantioselective Mn(salen) Epoxidation Catalysts, *Chem. Eur. J.* 1996, 2, 974-980) resulted in rearrangement and oxidation products (indicated by ¹H-NMR and TLC analyses). Use of protecting groups for the phenol offered no improvement and the absence of a protecting group resulted in apparent polymerization.

In the case of combretastatin A-4, the formation of the oxirane by the Jacobsen oxidation was observed by ¹H-NMR, but the compound resisted isolation. In addition, the derived 1,1-diphenylacetaldehyde and benzophenone **3a** products appeared to be formed. Following isolation of silyl ether **3a** and mass spectral analysis of the suspected benzophenone (10% yield, **3b**) cleavage of its silyl ether protective group showed the loss of one carbon atom. A conjugated carbonyl peak at 1633 cm⁻¹ was seen in the IR spectrum. Loss of the olefin bridge was established by NMR. This result suggested the need for structure confirmation of ketone **3b** by X-ray crystallography, which demonstrated the compound was 3-hydroxy-3',4,4',5'-tetramethoxybenzophenone (Figure 2). Finally, oxidative cleavage gave the silylated benzophenone **3a**.

Because of the potent cancer cell line growth inhibition displayed by the deprotected benzophenone, it was denominated "phenstatin." The structure

of phenstatin **3b** proved to be closely related to the aromatic system of podophyllotoxin **5**, a target of our efforts (Pettit, et al., Antineoplastic Agents V. The Aromatic System of Podophyllotoxin (Part B), *J. Med. Pharm. Chem.* 1962, 5, 800-808), which began in 1958, to locate the structural core responsible for the antineoplastic activity of this important lignan.

In order to improve the availability of phenstatin by a more efficient synthesis, several alternative routes were explored. The general procedure reported (Pettit et al., 1962, *supra*) in 1962 for obtaining ketone **4a** based on a morpholine amide intermediate proved to be most effective. The phenstatin amide precursor was prepared from 3-(*tert*-butyldimethylsilyl)oxy-4-methoxybenzaldehyde by oxidation to the corresponding carboxylic acid using potassium permanganate, followed by conversion to the acid chloride, and finally treatment with morpholine (Scheme 2, Figure 4). The amide **6a** was allowed to react with the lithium derivative prepared from 3,4,5-trimethoxybromobenzene (See: Jung, et al., Stereoselective Synthesis of an Analogue of Podophyllotoxin by an Intramolecular Diels-Alder Reaction, *J. Org. Chem.* 1985, 50, 1087-1105) and *t*-butyllithium at -78°C followed by deprotection to afford phenstatin **3b** in 30% overall yield. Similarly, a series of related **4** benzophenones were synthesized for SAR purposes as shown in Table 1 below.

Table 1. Physical Properties of the Amides and Benzophenones.

Compound	% Yield (from 5)	cryst. solvent	MP (°C)	Formula (c) (d)
6a	94	hexane	66-67	C ₁₈ H ₂₉ NO ₄ Si
6b	88	(a)	87-88	C ₁₃ H ₁₇ NO ₂
6c	73	(a)	78-79	C ₁₃ H ₁₇ NO ₄
6d	78	(a)	84-85	
6e	97	(a)	78-79	C ₁₃ H ₁₇ NO ₄
6f	100 (g)	(a)	117-119	C ₁₁ H ₁₁ Cl ₂ NO ₂

Compound	% Yield (from 5)	cryst. solvent	MP (°C)	Formula (c)(d)
6g	97 (g)	(b)	68-70	C ₁₁ H ₁₁ F ₂ NO ₂
3a	76	hexane	74-75	C ₂₃ H ₃₂ O ₆ Si
3b	83	ethyl acetate- hexane	149-150	C ₁₇ H ₁₈ O ₆
3c	72 (e)	n/a	clear oil at r.t.	C ₃₁ H ₃₁ O ₉ P
3d	(f)	water-acetone	165-167	C ₁₇ H ₁₇ O ₉ Na ₂ P
3e	93 (h)	ethyl acetate- hexane	164-165	C ₁₉ H ₂₀ O ₇
4a	98	methanol	124-125	C ₁₇ H ₁₆ O ₆
4b	75	hexane	104-105	C ₁₈ H ₂₀ O ₄
4c	53	toluene- hexane	120-122	C ₁₈ H ₂₀ O ₆
4d	70	hexane	121-122	C ₁₈ H ₂₀ O ₆
4e	34	hexane	131-132	C ₁₆ H ₁₄ Cl ₂ O ₄
4f	36	hexane	121-123	C ₁₆ H ₁₄ F ₂ O ₄

Code: (a)=crystallized following concentration of the flash column chromatography solvent. (b)=crystallized at 0°C upon isolation. (c)=All compounds were subjected to combustion analysis for C and H (and for Cl, F, and N where appropriate). The results were within $\pm 0.4\%$ of the calculated values. (d)=The ¹H- and ¹³C-NMR data at 300 MHz was also consistent with the assigned structure. (e)=From **3b**. (f)=from **3c**. (g)=% Yield from acid chloride. (h)=% yield from phenstatin.

Because of the improved therapeutic effects of the combretastatin A-4 sodium phosphate **1d** prodrug (Pettit, et al., Antineoplastic Agents 322. Synthesis of Combretastatin A-4 Prodrugs, *Anticancer Drug Design* 1995, 10, 299-309) when compared to the parent phenol **1b** (Pettit, et al., Isolation and Structure of the Strong Cell Growth and Tubulin Inhibitor Combretastatin A-4, *Experientia* 1989, 45, 209-211), the corresponding phenstatin prodrug **3d** was synthesized (**3b**→**3d**, Scheme 3, Figure 5). Both were used in the previous

phosphorylation techniques (Pettit, et al., Antineoplastic Agents 322., *Anticancer Drug Design* 1995, *supra*; and Pettit, et al., Antineoplastic Agents 320. Synthesis of a Practical Pancratistatin Prodrug, *Anticancer Drug Design* 1995, 10, 243-250) for such syntheses, based on pentavalent and trivalent phosphorous precursors. However, they proved to be substantially less effective than the dibenzylphosphite approach (Silverberg, et al., A Simple, Rapid and Efficient Protocol for the Selective Phosphorylation of Phenols with Dibenzyl Phosphite, *Tetrahedron Lett.* 1996, 37, 771-774). The prodrug was synthesized in 3 steps from phenstatin *via* phosphorylation of phenol **3b** employing dibenzylphosphite (under basic conditions in 1:1 acetonitrile - DMF) (*Id.*), followed by cleavage of the benzyl groups **3c** *via* catalytic hydrogenolysis (Pettit, et al., Antineoplastic Agents 320., *Anticancer Drug Design* 1995, *supra*) and reaction of the phosphoric acid product with sodium methoxide in methanol to afford sodium phosphate prodrug **3d** in 56% overall yield.

Accordingly, the prime object of the present invention is to provide an economically viable method for the synthesis of phenstatin and prodrugs thereof which are water soluble and can be used in the preparation of useful antineoplastic preparations.

These and still further objects as shall hereinafter appear are readily fulfilled by the present invention in a remarkably unexpected manner as will be readily discerned from the following detailed description of an exemplary embodiment thereof especially when read in conjunction with the drawings attached hereto.

FIG. 1 is a display of the graphic structures of compounds 1-8 and selected derivatives thereof (as identified in the specification);

FIG. 2 is a computer generated perspective drawing of phenastatin (**3b**);

FIG. 3 is a flow diagram showing the oxidation of Combretastatin A-4 silyl ether (**1c**) to phenstatin silyl ether (**3a**) (herein "Scheme 1");

FIG 4 is a flow diagram of the sequences herein referred to as "Scheme 2"; and

FIG. 5 is a flow diagram showing the conversion of phenstatin (**3b**) to phenstatin prodrug (**3d**) (as identified as "Scheme 3" in the specification).

Compounds **3b**, **3d**, **1d**, and **4a-f** were comparatively evaluated in the NCI 60 cell-line human tumor screen (Boyd, Status of the NCI preclinical antitumor drug discovery screen. Implications for selection of new agents for clinical trial, *CANCER: Principles and Practices of Oncology Updates*, DeVita, et al. (Eds.), Lippincott: Philadelphia, 1989; Vol. 10, No. 3, pp. 1-12; Boyd, The Future of New Drug Development. Section I. Introduction to Cancer Therapy, *Current Therapy in Oncology*, Niederhuber, (Ed.), B.C. Decker, Inc.: Philadelphia, 1993; pp. 11-22; Boyd, et al., Data display and analysis strategies from NCI disease-oriented *in vitro* antitumor drug screen. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*, Valeriote, et al. (Eds.), Kluwer Academic Publishers: Amsterdam, 1992; pp. 11-34; Stinson, et al., Morphological and Immunocytochemical Characteristics of Human Tumor Cell Lines for Use in a Disease-Oriented Anticancer Drug Screen, *Anticancer Res.* 1992, 12, 1035-1054; Boyd, et al., Some Practical Considerations and Applications of the NCI *in vitro* Drug Discovery Screen, *Drug Dev. Res.* 1995, 34, 91-109). As illustrated in Table 2, below, the water soluble phenstatin phosphate prodrug **3d** and the parent phenstatin **3b** were essentially indistinguishable in potency (e.g., mean panel GI₅₀ values) and differential cytotoxicity profiles (e.g., Compare correlation coefficients). Moreover, **3d** and **3b** were quite similar to the combretastatin A-4 phosphate prodrug **1d** both in terms of potency and differential cytotoxicity. In contrast, the related benzophenones **4a-f** were generally much less potent than **3b**, **3d** or **1d**, although their differential cytotoxicity profiles (and therefore their presumed mechanism of action (Boyd, et al., 1995, *supra*)) did not differ remarkably from

the lead compound. Interestingly, the acetate derivative **3e** of phenstatin exhibited significant human cancer cell line inhibitory activity: e.g., CNS U251 GI₅₀ 0.0039 $\mu\text{g/ml}$, pharynx FADU GI₅₀ 0.0055 $\mu\text{g/ml}$ and prostate DU-145 GI₅₀ 0.0029 $\mu\text{g/ml}$.

Table 2. Comparative Evaluations of Phenstatin **3b**, Phenstatin Prodrug **3d**, Combretastatin A-4 Prodrug **1d** and Other Related Benzophenones **4a-f** in the NCI 60 Cell-Line Human Tumor Screen^a

Compound	Mean Panel GI ₅₀ (\pm S.D) ^b[x 10 ⁻⁸ M]	COMPARE Correlation Coefficient ^b
3b	6.01 (3.76)	1.00
3d	7.33 (4.54)	0.94
1d	1.28 (0.44)	0.81
4a	270. (21.4)	0.74
4b	348. (45.4)	0.79
4c	511. (52.5)	0.74
4d	54.3 (4.94)	0.80
4e	512. (69.8)	0.73
4f	494. (99.0)	0.75

^aCompounds were tested in triplicate in the NCI screen as described (Boyd, 1993, *supra*; Boyd, et al., 1995, *supra*; Weinstein, et al., An Information-Intensive Approach to the Molecular Pharmacology of Cancer, *Science* 1997, 275,-343-349) in each of three concentration ranges (HITEST (Boyd, et al., 1995, *supra*) 10⁻⁵, 10⁻⁶, and 10⁻⁷M) using five, 10-fold dilutions within each range. ^bDescriptions and methods of calculation are described elsewhere (*Id.*). COMPARE analyses were performed using the mean graph profiles (*Id.*) of phenstatin **3b** as the seed.

Because of the known potent interactions of combretastatin A-4 **1b** (Pettit, et al., Antineoplastic Agents 291., *J. Med. Chem.* 1995, *supra*) and ketone **3f** (Cushman, et al., Synthesis and Evaluation of Analogues of (Z)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents, *J. Med. Chem.* 1992, 35, 2293-2306) with the colchicine site

of tubulin and because of COMPARE studies, such as those shown in Table 2, which suggested similar mechanisms of action, phenstatin **3b**, its prodrug **3d**, and compounds **4a-4f** were evaluated with others for potential inhibitory effects on tubulin polymerization and on the binding of colchicine to tubulin (Table 4, below).

In addition, comparative Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer cell growth inhibitor for phenstatin **3b** vs the phenstatin prodrug **3d** and the combretastatin A4 prodrug **1d** were conducted and are reported in Table 3, below.

Table 3. Comparative Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer Cell Growth Inhibition for Phenstatin **3b** vs. the Phenstatin Prodrug **3d** and the Combretastatin A-4 Prodrug **1d**.

Cell Type	Cell Line GI ₅₀ µg/ml	Phenstatin 3b	Phenstatin Prodrug 3d GI ₅₀ µg/ml	Combretastatin A-4 Prodrug 1d
Leukemia	P388	0.0033	<0.001	0.0004
Ovarian	OVCAR-3	0.0023	0.0025	0.023
CNS	SF-295	0.052	0.012	0.036
Renal	A498	0.38	0.05	0.041
Lung-NSC	NCI-H460	0.0057	0.035	0.029
Colon	KM20L2	0.04	0.27	0.34
Melanoma	SK-MEL-5	0.0038	0.0047	0.041

Simultaneous studies were performed for comparison of the new compounds with combretastatin A-4 **1b**, its prodrug **1d**, and the related compounds **3f** (Cushman, et al., 1992, *supra*), **1e** (Cushman, et al., Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential

Anticancer Agents That Inhibit Tubulin Polymerization, *J. Med. Chem.* 1991, *34*, 2579-2588), **7a** (Getahun, et al., Synthesis of Alkoxy-Substituted Diaryl Compounds and Correlation of Ring Separation with Inhibition of Tubulin Polymerization: Differential Enhancement of Inhibitory Effects under Suboptimal Polymerization Reaction Conditions, *J. Med. Chem.* 1992, *35*, 1058-1067), **7b** (Cushman, et al., 1992, *supra*), **8a** (Getahun, et al., 1992, *supra*), and **8b** (Cushman, et al., 1991, *supra*).

Table 4. Inhibition of tubulin polymerization and colchicine binding by phenstatin, combretastatin A-4, and related compounds.^a

Compound	Inhibition of tubulin polymerization IC ₅₀ , $\mu\text{M} \pm \text{SD}^b$	Inhibition of colchicine binding % inhibition
1b	1.2 ± 0.1	97
1d	> 80	4
1e	1.3 ± 0.2	97
3b	1.0 ± 0.2	86
3d	21 ± 3	37
3e	3.5 ± 0.5	71
4a	15 ± 2	0
4b	11 ± 2	7
4c	15 ± 2	1
4d	3.6 ± 0.8	58
4f	39 ± 7	0
7a	3.3 ± 0.5	65
7b	4.4 ± 0.2	56
8a	2.1 ± 0.3	77
8b	3.2 ± 0.3	54

^aReaction conditions described in detail in the Experimental Section.

^bSD, standard deviation.

Consistent with the relative antiproliferative activities summarized in Table 4, phenstatin **3b** was equivalent to combretastatin A-4 **1b** in its interactions with tubulin in the assays used here. Confirming unpublished observations with other bulky substituents on the B ring hydroxyl, the combretastatin A-4 prodrug **1d** was totally inactive against purified tubulin, but,

unexpectedly, the phenstatin prodrug **3d** had weak activity in inhibiting assembly and moderate activity as an inhibitor of colchicine binding. In the latter assay the prodrug was about 40% as active as phenstatin.

Previous structure-activity studies (Cushman, et al., *J. Med. Chem.* 1992, *supra*; Getahun, et al., *J. Med. Chem.* 1992, *supra*; Cushman, et al., *J. Med. Chem.* 1991, *supra*) had demonstrated maximal activity with tubulin with a 2-carbon bridge in the *Z*-stilbene configuration, whether or not the B ring hydroxyl was present, and the data of Table 4 reiterate this conclusion. The tetramethoxy ketone **3f** has shown increased activity relative to the analogous diphenylmethane **7b** (Cushman, et al., *J. Med. Chem.* 1992, *supra*), but the recovery of activity versus the *Z*-stilbene **1e** was incomplete. In contrast, in the presence of the B ring hydroxyl, the reduced activity of the diphenylmethane **7a** was completely recovered in the ketone phenstatin **3b**.

In terms of the effect of the B ring hydroxyl on interactions with tubulin, no significant difference was observed between the *Z*-stilbenes **1b** and **1e**, slight differences between the diphenylmethanes **7a** and **7b** and the dibenzyls **8a** and **8b**, and a small difference between the ketones **3b** and **3f**. When a difference was observed, it was always the phenol that had the greater activity.

Manipulation of molecular models demonstrated that it was possible to closely superimpose the two phenyl rings in the ketones with those in the *Z*-stilbenes, but this was also possible with the bibenzyls. This may indicate that tubulin specifically recognizes the sp^2 hybridization introduced with the carbonyl and stilbene bridges or the ring conjugation, analogous to that in colchicine, that these two bridges permit. The greater activity of **3b** relative to **3f** may also indicate that the B ring phenol group imposes steric constraints on the relative positions of the two phenyl rings.

The series of substituent modifications represented by compounds **4a-4f** failed to yield a derivative superior to **3b** as an inhibitor of either cell growth

or tubulin polymerization. Compound **4a**, which is closely analogous to the A, B, and E rings of podophyllotoxin **5**, had only weak activity against tubulin, and this was comparable to that obtained with **4c**. Compound **4c** has vicinal methoxy groups, with the hydroxyl group of **3b** replaced by a methoxyl group, instead of the methylenedioxy bridge of **4a**. The best activity in this group of compounds was observed with di-*meta* methoxy groups in ring B **4d**, but this compound was 3-4-fold less potent than phenstatin in inhibiting tubulin polymerization. There was a further 3-fold drop in activity with di-*meta* methyl groups in ring B **4b**, and compounds with halogens at these positions **4e** and **4f** were almost inert with tubulin. In the presence of a *para* methoxy group, the bulkiness of the substituent and/or the presence of an oxygen in the *meta* position of ring B seems to be critical for optimum inhibitory activity against tubulin in the ketone derivatives. A hydrogen **3f** leads to greater inhibition than a methoxy or phosphate group **4c**, **3d** but is less effective than a hydroxyl **3b**.

Based on the ring substituent structural modifications of phenstatin described herein, both the sp^2 hybridization of the carbonyl carbon that preserves the relative *cis*-relationship of the aromatic rings and the specific 3-hydroxy-4-methoxy-substitution appear necessary for strong cancer cell growth inhibition. Further development of phenstatin and its prodrug are in progress.

General Experimental Procedures. Ether (anhydrous diethyl ether), tetrahydrofuran (distilled from sodium and benzophenone), thionyl chloride and morpholine were distilled prior to use. Reagents were obtained from Sigma-Aldrich Co. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate unless otherwise noted. Flash column chromatography was performed using silica gel (230-400 mesh) and hexane-ethyl acetate as eluant. ANALTECH silica gel GHLF plates were used for TLC. All compounds were visible with fluorescent short-wave light (254 nm).

Melting points were recorded employing an ELECTROTHERMAL 9100 apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded by means of a VARIAN VXR-300 instrument and referenced to chloroform. ^{31}P -NMR was obtained from a VARIAN VXR-500 and referenced to an external standard (85% aqueous H_3PO_4). Mass spectral data were recorded using a VARIAN MAT 312 instrument (EIMS), and IR spectra were determined with a MATTONSON INSTRUMENTS 2020 GALAXY SERIES FTIR instrument. X-ray crystal structure data collection was performed on an ENRAF-NONIUS CAD4 diffractometer.

Jacobsen Oxidation of Combretastatin A-4 **1b**

To a mixture of 4-phenylpyridine-N-oxide (0.127g, 0.74 mmol) and (R,R)-(-)-[N,N'-bis(3,5-di-*tert*-butylsalicylidene)-1.2-cyclohexanediaminoat(2-)]manganese(III) chloride (Palucki, et al., Highly Enantioselective, Low-Temperature Epoxidation of Styrene, *J. Am. Chem. Soc.* 1994, 116, 9333-9334) (0.024g, 0.037 mmol) in a 50 mL flask was added a solution of 3'[(*tert*-butyldimethylsilyl)oxy]combretastatin A-4 (**1c**, 0.80g, 1.86 mmol) in 3 ml of ethyl-acetate (or methylene chloride). The mixture was stirred at 4°C for 20 minutes followed by addition of an aqueous 0.50 M NaOCl solution (6.32 mL, 3.16 mmol) *via* syringe. The temperature remained at 4°C, and reaction progress was monitored by TLC (4:1 hexane-EtOAc). After 5 h, the reaction mixture was warmed to room temperature, and the aqueous phase removed. The organic layer was washed successively with water (2 x 10 mL) and brine (10 mL) and dried over anhydrous Na_2SO_4 . Filtration and solvent removal *in vacuo* gave a brown residue that was subjected to flash column chromatography (4:1 hexane-ethyl acetate, 1" x 12" column). The silyl ether protected benzophenone **3a** ($R_f = 0.27$) was collected as a yellow oil. A second flash column chromatographic separation was necessary for final purification. Consistent 10% yields were obtained based on conversion of combretastatin A-4 silyl ether to phenstatin (**3b**, see below).

Phenstatin 3b.

Silyl ether **3a** (4.90 g, 11.3 mmol) was dissolved in dry tetrahydrofuran (100 mL), and 1.0 M tetrabutylammonium fluoride (11.3 mL, 11.3 mmol) was added (slowly via syringe) while rapidly stirring under Ar (Pietikäinen, *Tetrahedron Lett.* 1995, *supra*). After 15 min, ice (20 g) and ether (100 mL) were added successively. The ethereal layer was washed with water (3 x 100 mL), dried, filtered, and solvent removed under reduced pressure to yield a yellow solid. Flash chromatography (eluant: 3:2 hexane-ethyl acetate, Rf=0.17) was performed and afforded an off-white solid that recrystallized from hexane as colorless granules: EIMS *m/z* (peak height) 318 (M⁺, 100%), 303 (14%), 195 (30%), 151 (36%). IR (Nujol, cm⁻¹) 1633 (C=O), 1604 (aromatic C=C); ¹H-NMR (CDCl₃) δH 7.406 (1H, dd, J_{6/2} 2.1 Hz, J_{6/5} 9.4 Hz, H-6), 7.362 (1H, d, J_{2/6} 2.1 Hz, H-2), 7.011 (2H, s, H-2', 6'), 6.906 (1H, d, J_{5/6} 8.4 Hz, H-5), 5.662 (1H, s, -OH), 3.969 (3H, s, 4'-OCH₃), 3.913 (3H, s, 4-OCH₃), 3.860 (6H, s, 3', 5'-OCH₃); ¹³C-NMR (75.5 MHz, CDCl₃, assignments deduced with assistance from an APT spectrum) δC 193.3 (C=O), 152.8 (C-3',5'), 150.2 (C-4), 145.3 (C-3), 141.7 (C-4'), 133.2 (C-1'), 131.1 (C-1), 123.6 (C-6), 116.2 (C-2), 110.0 (C-5), 107.5 (C-2',6'), 60.9 (4-OCH₃), 56.3 (3',5'-OCH₃), 56.1 (4'-OCH₃).

Phenstatin acetate 3e.

Acetic anhydride (74 μL, 0.79 mmol) was added to a solution of phenstatin (**3b**, 0.2 g, 0.63 mmol), DMAP (7.8 mg, 0.063 mmol) and triethylamine (0.13 mL, 0.94 mmol) in dry methylene chloride (1.5 mL, under argon at room temperature). The yellow solution was stirred for 30 min (reaction was complete by TLC). Methanol (5 mL) was added and the reaction mixture concentrated to a solid that was washed with diethyl ether (3 x 10 mL) and partitioned between ethyl acetate and cold 1N hydrochloric acid. The organic layer was washed with 10% aqueous sodium bicarbonate and dried. Solvent removed, *in vacuo*, gave an off-white solid that was recrystallized from ethyl acetate-hexane to give colorless snowflakes of analytical purity: EIMS *m/z*

(peak height) 360 M⁺, 60%), 318 (100%), 303 (10%), 195 (20%), 151 (30%), 91 (32%), 44 (25%). ¹H-NMR (CDCl₃) δ H 7.740 (1H, dd, J_{6/2} 2.1 Hz, J_{6/5} 8.1 Hz, H-6), 7.556 (1H, d, J_{2/6} 2.1 Hz, H-2), 7.026 (1H, d, J_{5/6} 8.0 Hz, H-5), 7.013 (2H, s, H-2', 6'), 3.914 (3H, s, 4-OCH₃), 3.907 (3H, s, 4'-OCH₃), 3.863 (6H, s, 3', 5'-OCH₃), 2.315 (3H, s, -OCOCH₃).

X-Ray Crystal Structure Determination of Phenstatin 3b.

Colorless crystals of ketone **3b** were grown as clusters of thick plates from an ether solution. Cleavage of a single crystal fragment from one of the clusters provided a usable specimen (0.54 x 0.32 x 0.28 mm), which was mounted on the end of a glass capillary tube. Data collection was performed at 25 ± 1°C. Crystal data: C₁₇H₁₈O₆, monoclinic space group P2₁/c with a = 12.608(2), b = 14.858(2), c = 8.738(3) Å, β = 104.69(2)°, V = 1583.3(12) Å³, λ(CuKα) = 1.54178 Å, ρ₀ = 1.299 g cm⁻³, ρ_c = 1.335 g cm⁻³ for Z=4 and F.W. = 318.31, F(000)=672. The structure determined for **3b** converged to the standard crystallographic residual of R_i = 0.0462 for 2453 reflections in which Fo > 4.0 sigma(Fo) and 0.0480 for all 2610 reflections. The corresponding wR2 Sheldrick values, based on Fo², were 0.1454 and 0.1475, respectively. The Goodness of Fit for all data was 1.042. Final bond distances and angles were all within acceptable limits.

3-(tert-butyldimethylsilyl)oxy-4-methoxybenzoic acid.

To a solution of 3-[tert-butyldimethylsilyl]oxy-4-methoxy-benzaldehyde² (35.0 g, 131 mmol) in acetone (400 mL) was added (stirring) a warm solution (40°C) of potassium permanganate (35.3 g, 223 mmol) in water (50 mL)-acetone (250 mL) over 30 min. The reaction mixture was stirred for 45 min (complete by TLC analysis). The suspension was cooled and filtered through a pad of celite, and the filtrate concentrated. The residue was dissolved in ethyl acetate (1000 mL) and transferred to a separatory funnel containing 1 N HCl (800 mL). The organic layer was extracted with water (5 x 1000 mL), brine (500 mL), dried (sodium sulfate), and solvent removed *in vacuo* to give

a crystalline powder. Recrystallization from hexane afforded the benzoic acid as colorless needles (31.0 g, 84% yield): mp=163.5-164.5°C; EIMS m/z (peak height) 282 (M^+), 267 (2%), 225 (92%), 210 (100%), 195 (20%); IR (Nujol, cm^{-1}) 3100 (broad O-H stretch), 1682 (C=O), 1681 (aromatic C=C); 1H -NMR ($CDCl_3$) δ H 7.732 (1H, dd, $J_{6/2}$ 2.2 Hz, $J_{6/5}$ 8.4 Hz, H-6), 7.569 (1H, d, $J_{2/6}$ 1.2 Hz, H-2), 6.894 (1H, d, $J_{5/6}$ 8.5 Hz, H-5), 3.882 (3H, s, -OCH₃), 1.009 (9H, s, -Ot-Bu), 0.174 (6H, s, -Si(CH₂)₂-); ^{13}C -NMR (75.5 MHz, $CDCl_3$, assignments assisted by an APT spectrum) δ C 172.9 (C=O), 156.6 (C-4), 145.4 (C-3), 125.7 (C-2), 123.0 (C-6), 122.4 (C-1), 111.5 (C-5), 55.8 (-OCH₃), 25.9 (-CH₃), -4.5 (-Si(CH₂)₂-), -4.5 (-Si-C-).

General Procedure for Synthesis of Morpholine Amides 6a-g.

To a 1.0 M solution of the carboxylic acid in chloroform at room temperature was added 2 equivalents of thionyl chloride. The solution was heated to reflux under Ar until TLC (3:1 hexane-ethyl acetate) showed no starting material (approximately 4-8 h). The solution was concentrated to an oil that solidified under high vacuum (0.05 mm Hg), and the crude acid chloride was used in the next step without purification. Two equivalents of morpholine were slowly injected *via* syringe over a 5 min period to a 0.3 M solution of the acid chloride in toluene (under Ar). The reaction mixture was stirred at room temperature until the starting acid chloride was consumed (approx. 1.5-4 h, according to TLC using 3:1 hexane-ethyl acetate). The solution was filtered through a pad of celite to remove morpholine hydrochloride, and the pad washed with toluene. The filtrate was concentrated to an oil that was subjected to flash column chromatography. Concentration of the respective fractions led to the respective amides (Table 1). **6f** and **6g** were synthesized from the acid chloride.

N-[(3-(*tert*butyldimethylsilyl)oxy-4-methoxybenzoyl)morpholine 6a.

A cotton-like solid followed chromatography (eluant: hexane-ethyl acetate 3:2 1:4) and recrystallization from hexane (Table 1). EIMS m/z (peak

height) 351 (M^+), 336 (4%), 294 (100%), 278 (26%), 265 (8%), 193 (70%), 165 (16%).

N-(3,5-dimethylbenzoyl)morpholine 6b.

After chromatography (3:2 hexane-ethyl acetate, $R_f=0.19$), a fluffy-appearing solid was obtained (Table 1).

N-(3,4-dimethoxybenzoyl)morpholine 6c.

Amide **6c** was obtained as a colorless flaky solid (Gardner, et al., The Polyoxyphenols of Western Red Cedar (*Thuja Plicata* Donn), *Can. J. Chem.* 1960, 38, 2387-2394) following chromatography (1:2 hexane-ethyl acetate, $R_f=0.16$).

N-(3,4-methylenedioxybenzoyl)morpholine 6d.

Amide **6d** (Kasztreiner, et al., Synthesis and Pharmacological Investigation of New Alkoxybenzamides II, *Biochem. Pharmacol.* 1962, 11, 651-657) was isolated as a colorless flaky solid following chromatography (1:1 hexane-ethyl acetate, $R_f=0.22$).

N-(3,5-dimethoxybenzoyl)morpholine 6e.

After chromatographic (1:1 hexane-ethyl acetate $R_f=0.18$) purification amide **6e** was collected as a colorless solid. TOF m/z / 251.6 (M^+).

N-(3,5-dichlorobenzoyl)morpholine 6f.

The clear oily product crystallized at 0°C: EIMS m/z (peak height) 259 (M^+), 261 ($M+2$), 263 ($M+4$), 258 (M^+-H), 175 (62%), 173 (96%), 86 (76%), 56 (100%).

N-(3,5-difluorobenzoyl)morpholine 6g.

Amide **6g** ($R_f=0.21$ in 3:2 hexane-ethyl acetate) gave EIMS m/z (peak height) 227 (M^+), 141 (100%), 113 (50%), 86 (36%), 56 (66%).

General Procedures for Synthesis of Benzophenones 3a, 4a-f

A flame-dried flask containing a 0.1 M solution of 3,4,5-trimethoxybromobenzene (1.1 eq in dry tetrahydrofuran) was cooled to -78°C, evacuated to 1 Torr and refilled with Ar for 10 cycles. To this solution was slowly added *tert*-butyllithium (2.2 eq), and the reaction mixture was stirred for

15 min. A second dry flask containing a 0.1 M solution of the morpholine amide (1.0 eq) in tetrahydrofuran was cooled to -78°C, evacuated, and refilled with Ar. The phenyllithium reagent was transferred *via* cannula to the amide solution. The reaction mixture was stirred at -65 to -78°C for 4 h, followed by a slow warming to room temperature. Progress of the reaction was monitored by TLC (hexane-ethyl acetate). The reaction was stopped by adding 6 eq of isopropyl alcohol and stirring for 1 h. Water was added, and the mixture was extracted with ether (3x). The ethereal extracts were combined, washed with water, dried, and solvent removed to give an oil that was subjected to flash chromatography. Collection and concentration of the required fractions afforded the benzophenone (Table 1).

3-(*tert*-butyldimethylsilyl)oxy- 3',4,4',5'-tetramethoxybenzophenone 3a.

Using amide **6a** (0.20 g, 0.57 mmol), 3,4,5-trimethoxybromobenzene (0.169 g, 0.683 mmol), and t-BuLi (0.74 mL, 1.25 mmol) the above scheme led to crude phenstatin silyl ether **3a** (0.199 g, R_f=0.29 in 4:1 hexane-ethyl acetate). Purification and recrystallization from hexane afforded large glass-like prisms (Table 1); EIMS *m/z* (peak height) 432 (M⁺), 417 (2%), 402 (1%), 375 (100%), 360 (58%), 345 (4%), 193 (26%).

3,4,5-trimethoxy-3',4'-methylenedioxybenzophenone 4a.

Flash column chromatography (4:1 hexane-ethyl acetate R_f=0.20) and recrystallization from methanol afforded small colorless needles (Pettit, et al., *J. Med. Pharm. Chem.* 1962, *supra*).

3,4,5-trimethoxy-3',5'-dimethylbenzophenone 4b.

Chromatography (9:1 hexane-ethyl acetate R_f=0.19) and recrystallization from hexane yielded off-white needles: EIMS *m/z* (peak height) 300 (M⁺, 100%), 285 (14%), 270 (4%), 375 (100%), 195 (40%).

3,3',4,4',5-pentamethoxybenzophenone 4c.

Purification by column chromatography (4:1 hexane-ethyl acetate R_f=0.10) and recrystallization from toluene-hexane gave an off-white powder:

EIMS m/z (peak height) 332 (M^+ , 100%), 317 (6%), 301 (8%), 195 (16%), 165 (22%).

3,3',4,5,5'-pentamethoxybenzophenone 4d.

After recrystallization from hexane, the off-white powder did not require chromatographic purification: EIMS m/z (peak height) 332 (M^+ , 100%), 317 (6%), 301 (8%), 195 (16%), 165 (22%).

3,5-dichloro-3',4',5'-trimethoxybenzophenone 4e.

Chromatographic (9:1 hexane-ethyl acetate, $R_f=0.28$) separation and recrystallization from hexane afforded clear diamond-shaped crystals: EIMS m/z (peak height) 340 (M^+ , 100%), 342 ($M+2$), 344 ($M+4$), 325 (26%), 310 (4%), 195 (46%).

3,5-difluoro-3',4',5'-trimethoxybenzophenone 4f.

Following chromatography (9:1 hexane-ethyl acetate, $R_f=0.21$) and recrystallization from hexane, the ketone was obtained as colorless needles: EIMS m/z (peak height) 318 (M^+ , 100%), 293 (20%), 278 (4%), 195 (22%), 141 (36%), 113 (16%).

3-O-Dibenzylphosphoryl-phenstatin 3c.

To a dry flask equipped with septum, magnetic stirrer, thermometer and Ar inlet containing dry acetonitrile (50 mL) and DMF (50 mL) was added phenstatin (**3b**, 2.0 g, 6.28 mmol). Upon cooling to -10°C , bromotrichloromethane (3.10 mL, 31.4 mmol) was added followed by triethylamine (1.84 mL, 13.2 mmol) and dimethylaminopyridine (77 mg, 0.63 mmol). After one minute, dropwise addition of dibenzylphosphite was begun while maintaining a temperature of -7 to -10°C . The resultant yellow solution was monitored by TLC (1:1 hexane-ethyl acetate, $R_f=0.19$) until complete. At that time (1.5 h), 0.5 M aqueous KH_2PO_4 (50 mL) was added followed by extraction with ethyl acetate (3 x 100 mL). The organic extract was washed successively with water (200 mL), brine (150 mL), and dried (sodium sulfate). The solution was filtered and concentrated *in vacuo* to a milky oil. Flash

column chromatography (eluant: 1:1 hexane-ethyl acetate) was performed to give benzyl phosphate **3c** as a clear oil (2.60 g, 72% yield): EIMS m/z (peak height) (578, M^+), (486, 4%), (91, 100%). 1H -NMR ($CDCl_3$) δ H 7.694 (1H, d, $J_{6/5}$ 8.0 Hz, H-6), 7.657 (1H, s, H-2), 7.319 (10H, s, Ar-H), 7.025 (2H, s, H-2',6'), 7.000 (1H, d, $J_{5/6}$ 9.0 Hz, H-5), 5.182 (4H, d, J 8.5 Hz-O- CH_2 -Ph), 3.880 (9H, s, 3', 5', 4-O CH_3); ^{31}P -NMR ($CDCl_3$, decoupled, 202.35 MHz) δ P -5.22.

Disodium Phenstatin 3-O-phosphate **3d**.

The benzyl phosphate (**3c**, 0.68 g, 1.18 mmol) was added to anhydrous ethyl alcohol (denatured, 50 mL) in a dry flask equipped with a magnetic stirrer. After evacuating the vessel and refilling with Ar (two cycles), 5% palladium-on-carbon (0.58 g) was added. The flask was evacuated and refilled with H_2 to 10 psi (3x). The mixture was stirred vigorously for 15 min and filtered through a 1 cm pad of celite. The flask and celite were washed with ethanol and solvent was removed under reduced pressure to afford a foamy tan solid (0.44 g) that was used in the next step.

The deprotected phosphate (0.44 g, 1.10 mmol) was dissolved in anhydrous methanol (15 mL) and sodium methoxide (88 mg, 2.20 mmol) was added at room temperature. The cloudy solution was stirred for 20 hours and the reaction mixture was concentrated to a white solid that was washed with 2-propanol and recrystallized (3x) from water-acetone. The phenstatin prodrug (**3d**, 0.38g, 78% yield) was collected by filtration and found to give: LRFAB m/z 443.02 ($M+H^+$), calculated 443.048; EIMS m/z (peak height) 318 (100%, $M+PO_3Na_2+H$), 303 (12%), 195 (20%), 151 (23%); 1H -NMR (D_2O) δ H 7.644 (1H, t, $J_{2/6}$ 1.8 Hz, H-2), 7.366 (1H, dd, $J_{6/2}$ 1.8 Hz, $J_{6/5}$ 8.5 Hz, H-6), 7.006 (1H, d, $J_{5/6}$ 8.5 Hz, H-5), 6.933 (2H, s, H-2',6'), 3.844 (3H, s, 4'-O CH_3), 3.748 (6H, s, 3',5'-O CH_3) 3.734 (3H, s, 4-O CH_3); ^{13}C -NMR (D_2O , reference to $CDCl_3$) δ C 193.5 (C=O), 151.1; and ^{31}P -NMR (D_2O , decoupled, -202.35 MHz) δ P -1.965. The solubility of phenstatin prodrug **3d** was found to be 30 mg/mL in distilled water at 25°C.

Tubulin assays.

Electrophoretically homogeneous bovine brain tubulin was purified from bovine brain as described previously (Hamel, et al., Separation of active tubulin and microtubule-associated proteins by ultracentrifugation and isolation of a component causing the formation of microtubule bundles, *Biochemistry* 1984, 23, 4173-4184). Reaction mixtures (0.24 mL during preincubation, 0.25 mL during incubation, with all concentrations referring to the final volume) contained 0.8 M monosodium glutamate (taken from 2.0 M stock solution adjusted to pH 6.6 with HCl), 1.0 mg/mL (10 μ M) tubulin, 4% (v/v) dimethyl sulfoxide, and varying concentrations of drug. Samples were incubated for 15 min at 30°C and chilled on ice. GTP, required for polymerization, was added in 10 μ L to a final concentration of 0.4 mM. Samples were transferred to cuvettes held at 0°C by electronic temperature controllers in GILFORD model 250 recording spectrophotometers. Baselines were established, and the reaction was initiated by a jump (approximately 1 min) to 30°C. Extent of assembly after 20 min was the parameter used to determine IC₅₀ values. Active compounds were examined in at least three independent experiments, and inactive compounds were generally evaluated twice. In most experiments, four spectrophotometers were used, with two control reaction mixtures.

The binding of [³H]colchicine to tubulin was measured on DEAE-cellulose filters as described elsewhere (Kang, et al., *N*-acetylcolchicinol *O*-methyl ether and thiocolchicine, potent analogs of colchicine modified in the C ring: Evaluation of the mechanistic basis for their enhanced biological properties, *J. Biol. Chem.* 1990, 265, 10255-10259). Reaction mixtures contained 0.1 mg/mL (1.0 μ M) tubulin, 5.0 μ M [³H]colchicine, and 5.0 μ M inhibitor and were incubated for 10 min at 37°C.

Dosing.

The dosage administered will be dependent upon the identity of the neoplastic disease; the type of host involved, including its age, health and

weight; the kind of concurrent treatment, if any; the frequency of treatment and therapeutic ratio.

Illustratively, dosage levels of the administered active ingredients are: intravenous, 0.1 to about 20 mg/kg; intramuscular, 1 to about 50 mg/kg; orally, 5 to about 100 mg/kg; intranasal instillation, 5 to about 100 mg/kg; and aerosol, 5 to about 100 mg/kg of host body weight.

Expressed in terms of concentration, an active ingredient can be present in the compositions of the present invention for localized use about the cutis, intranasally, pharyngolaryngeally, bronchially, intravaginally, rectally, or ocularly in a concentration of from about 0.01 to about 50% w/w of the composition; preferably about 1 to about 20% w/w of the composition; and for parenteral use in a concentration of from about 0.05 to about 50% w/v of the composition and preferably from about 5 to about 20% w/v.

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions of suspensions, and oral solutions or suspensions and the like, containing suitable quantities of an active ingredient.

For oral administration either solid or fluid unit dosage forms can be prepared.

Powders are prepared quite simply by comminuting the active ingredient to a suitably fine size and mixing with a similarly comminuted diluent. The diluent can be an edible carbohydrate material such as lactose or starch. Advantageously, a sweetening agent or sugar is present as well as a flavoring oil.

Capsules are produced by preparing a powder mixture as hereinbefore described and filling into formed gelatin sheaths. Advantageously, as an adjuvant to the filling operation, a lubricant such as talc, magnesium stearate,

calcium stearate and the like is added to the powder mixture before the filling operation.

Soft gelatin capsules are prepared by machine encapsulation of a slurry of active ingredients with an acceptable vegetable oil, light liquid petrolatum or other inert oil or triglyceride.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and pressing into tablets. The powder mixture is prepared by mixing an active ingredient, suitably comminuted, with a diluent or base such as starch, lactose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as corn syrup, gelatin solution, methylcellulose solution or acacia mucilage and forcing through a screen. As an alternative to granulating, the powder mixture can be slugged, i.e., run through the tablet machine and the resulting imperfectly formed tablets broken into pieces (slugs). The slugs can be lubricated to prevent sticking to the tablet-forming dies by means of the addition of stearic acid, a stearic salt, talc or mineral oil. The lubricated mixture is then compressed into tablets.

Advantageously, the tablet can be provided with a protective coating consisting of a sealing coat or enteric coat of shellac, a coating of sugar and methylcellulose and polish coating of carnauba wax.

Fluid unit dosage forms for oral administration such as in syrups, elixirs and suspensions can be prepared wherein each teaspoonful of composition contains a predetermined amount of an active ingredient for administration. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, flavoring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic vehicle with suitable sweeteners together with a flavoring agent. Suspensions can be prepared of the insoluble forms with a suitable vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration, fluid unit dosage forms are prepared utilizing an active ingredient and a sterile vehicle, water being preferred. The active ingredient, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble active ingredient can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. Parenteral suspensions are prepared in substantially the same manner except that an active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

In addition to oral and parenteral administration, the rectal and vaginal routes can be utilized. An active ingredient can be administered by means of a suppository. A vehicle which has a melting point at about body temperature or one that is readily soluble can be utilized. For example, cocoa butter and various polyethylene glycols (Carbowaxes) can serve as the vehicle.

For intranasal instillation, a fluid unit dosage form is prepared utilizing an active ingredient and a suitable pharmaceutical vehicle, preferably P.F. water, a dry powder can be formulated when insufflation is the administration of choice.

For use as aerosols, the active ingredients can be packaged in a pressurized aerosol container together with a gaseous or liquified propellant, for example, dichlorodifluoromethane, carbon dioxide, nitrogen, propane, and the like, with the usual adjuvants such as cosolvents and wetting agents, as may be necessary or desirable.

5 The term "unit dosage form" as used in the specification and claims refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the novel unit dosage forms of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitation inherent in the art of compounding such an active material for therapeutic use in humans, as disclosed in this specification, these being features of the present invention. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, troches, suppositories, powder packets, wafers, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and other forms as herein described.

10
15 The active ingredients to be employed as antineoplastic agents can be easily prepared in such unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures. The following preparations are illustrative of the preparation of the unit dosage forms of the present invention, and not as a limitation thereof. Several dosage forms were prepared embodying the present invention. They are shown in the following examples in which the notation "active ingredient" signifies either phenstatin **3b** and/or phenstatin prodrug **3d**, and/or benzophenones **4a-f** or any other compound described herein.

25 COMPOSITION "A"

Hard-Gelatin Capsules

One thousand two-piece hard gelatin capsules for oral use, each capsule containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Corn Starch	20 g
Talc	20 g
Magnesium stearate	2 g

5 The active ingredient, finely divided by means of an air micronizer, is added to the other finely powdered ingredients, mixed thoroughly and then encapsulated in the usual manner.

 The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

10 Using the procedure above, capsules are similarly prepared containing an active ingredient in 50, 250 and 500 mg amounts by substituting 50 g, 250 g and 500 g of an active ingredient for the 200 g used above.

COMPOSITION "B"

Soft Gelatin Capsules

15 One-piece soft gelatin capsules for oral use, each containing 200 mg of an active ingredient, finely divided by means of an air micronizer, are prepared by first suspending the compound in 0.5 ml of corn oil to render the material capsulatable and then encapsulating in the above manner.

20 The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

COMPOSITION "C"

Tablets

25 One thousand tablets, each containing 200 mg of an active ingredient, are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Lactose	300 g
Corn starch	50 g
Magnesium stearate	4 g
Light liquid petrolatum	5 g

The active ingredient, finely divided by means of an air micronizer, is added to the other ingredients and then thoroughly mixed and slugged. The slugs are broken down by forcing them through a Number Sixteen screen. The resulting granules are then compressed into tablets, each tablet containing 200 mg of the active ingredient.

The foregoing tablets are useful for treating a neoplastic disease by the oral administration of one or two tablets one to four times a day.

Using the procedure above, tablets are similarly prepared containing an active ingredient in 250 mg and 100 mg amounts by substituting 250 g and 100 g of an active ingredient for the 200 g used above.

COMPOSITION "D"

Oral Suspension

One liter of an aqueous suspension for oral use, containing in each teaspoonful (5 ml) dose, 50 mg of an active ingredient, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	10 g
Citric acid	2 g
Benzoic acid	1 g
Sucrose	790 g
Tragacanth	5 g
Lemon Oil	2 g
Deionized water, q.s.	1000 ml

The citric acid, benzoic acid, sucrose, tragacanth and lemon oil are dispersed in sufficient water to make 850 ml of suspension. The active ingredient, finely divided by means of an air micronizer, is stirred into the syrup unit uniformly distributed. Sufficient water is added to make 1000 ml.

The composition so prepared is useful for treating a neoplastic disease at a dose of 1 teaspoonful (5 ml) three times a day.

COMPOSITION "E"

Parenteral Product

A sterile aqueous suspension for parenteral injection, containing 30 mg of an active ingredient in each milliliter for treating a neoplastic disease, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	30 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Water for injection, q.s. 1000 ml.	

All the ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is filled into sterile vials and the vials sealed.

The composition so prepared is useful for treating a neoplastic disease at a dose of 1 milliliter (1ml) three times a day.

COMPOSITION "F"

Suppository, Rectal and Vaginal

One thousand suppositories, each weighing 2.5 g and containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	15 g
Propylene glycol	150 g
Polyethylene glycol #4000, q.s.	2,500 g

The active ingredient is finely divided by means of an air micronizer and added to the propylene glycol and the mixture passed through a colloid mill until uniformly dispersed. The polyethylene glycol is melted and the propylene glycol dispersion is added slowly with stirring. The suspension is poured into

unchilled molds at 40°C. The composition is allowed to cool and solidify and then removed from the mold and each suppository foil wrapped.

The foregoing suppositories are inserted rectally or vaginally for treating a neoplastic disease.

COMPOSITION "G"

Intranasal Suspension

One liter of a sterile aqueous suspension for intranasal instillation, containing 20 mg of an active ingredient in each milliliter, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	15 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Deionized water, q.s.	1000 ml.

All the ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is aseptically filled into sterile containers.

The composition so prepared is useful for treating a neoplastic disease, by intranasal instillation of 0.2 to 0.5 ml given one to four times per day.

An active ingredient can also be present in the undiluted pure form for use locally about the cutis, intranasally, pharyngolaryngeally, bronchially, or orally.

COMPOSITION "H"

Powder

Five grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is placed in a shaker-type container.

The foregoing composition is useful for treating a neoplastic disease, at localized sites by applying a powder one to four times per day.

COMPOSITION "I"

Oral Powder

One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is divided into individual doses of 200 mg and packaged.

The foregoing powders are useful for treating a neoplastic disease, by the oral administration of one or two powders suspended in a glass of water, one to four times per day.

COMPOSITION "J"

Insufflation

One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer.

The foregoing composition is useful for treating a neoplastic disease, by the inhalation of 300 mg one to four times a day.

From the foregoing, it becomes readily apparent that a new and useful antineoplastic factor and new and useful antineoplastic preparations have been herein described and illustrated which fulfill all of the aforesated objectives in a remarkably unexpected fashion. It is of course understood that such modifications, alterations and adaptations as will readily occur to the artisan confronted with this disclosure are intended within the spirit of the present invention.

Claims

1. The method of synthesizing phenstatin comprising the steps of:
oxidizing 3-(tert-butyl dimethylsilyl)oxy-4-methoxybenzaldehyde with
potassium permanganate to form the corresponding carboxylic acid;

converting said carboxylic acid to the corresponding acid chloride;

treating said acid chloride with the lithium derivative obtained from 3,
4, 5-trimethoxybenzene and t-butyllithium to form a protected product;

and deprotecting said protected product to form phenstatin.

2. The method of synthesizing phenstatin prodrug comprising the steps of:

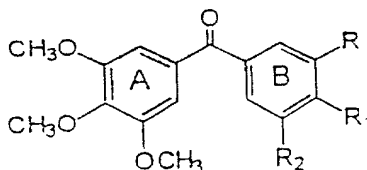
phosphorylating phenstatin with dibenzylphosphite in the presence of
bromodichloromethane to form a phosphate ester;

cleaving the benzyl groups from said phosphate ester by means of
catalytic hydrogenolysis; and

reacting the cleaved phosphate ester with sodium methoxide to produce
the phenstatin sodium phosphate prodrug.

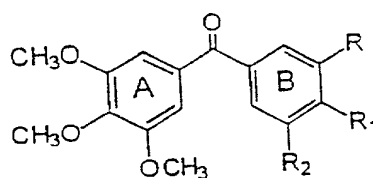
3. The method of inhibiting cancer cell growth and tubulin polymerization
in an environment inflicted therewith comprising: introducing into said
environment a pharmaceutically acceptable carrier and a small but effective
amount of phenstatin prodrug.

4. Phenstatin prodrugs and derivatives thereof having the structure:



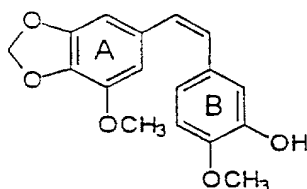
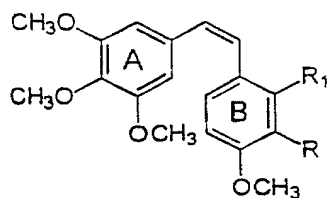
wherein when $R=H$ and $R_1 = OCH_3$, R_2 is OPO_3NA_2 , $OCOCH_3$, H , or OCH_3 and when $R=R_2$, R_2 is OCH_3 , CH_3 , Cl or F and R_1 is H and when $R_1=R_2$, R_2 is OCH_3 or OCH_2O and R is H .

5. The method of inhibiting human cancer cell growth in a host inflicted therewith comprising administering to said host in pharmaceutically acceptable carrier a small but effective amount of a compound selected from the group consisting of phenstatin, phenstatin prodrug and the derivatives thereof having the structure.

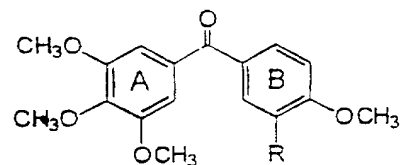


wherein when $R=H$ and $R_1 = OCH_3$, R_2 is OPO_3NA_2 , $OCOCH_3$, H , or OCH_3 and when $R=R_2$, R_2 is OCH_3 , CH_3 , Cl or F and R_1 is H and when $R_1=R_2$, R_2 is OCH_3 or OCH_2O and R is H .

1/5



2, Combretastatin A-2

1a, R = OH, R₁ = OH

Combretastatin A-1

1b, R = OH, R₁ = H

Combretastatin A-4

1c, R = OSi(CH₃)₂C(CH₃)₃, R₁ = H1d, R = OPO₃Na₂, R₁ = H

Combretastatin A-4 prodrug

1e, R = R₁ = H3a, R = OSi(CH₃)₂C(CH₃)₃

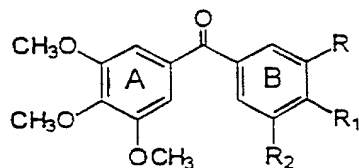
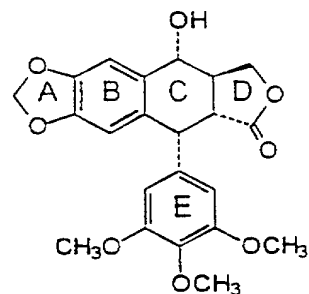
3b, R = OH, Phenstatin

3c, R = OPO₃(C₆H₅CH₂)₂3d, R = OPO₃Na₂

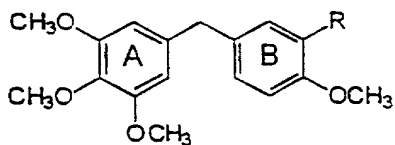
Phenstatin prodrug

3e, R = OCOCH₃

3f, R = H

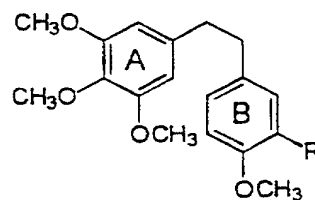
4a, R = H, R₁, R₂ = OCH₂O4b, R = R₂ = CH₃, R₁ = H4c, R = H, R₁ = R₂ = OCH₃4d, R = R₂ = OCH₃, R₁ = H4e, R = R₂ = Cl, R₁ = H4f, R = R₂ = F, R₁ = H

5



7a, R = OH

7b, R = H



8a, R = OH

8b, R = H

Figure 1.

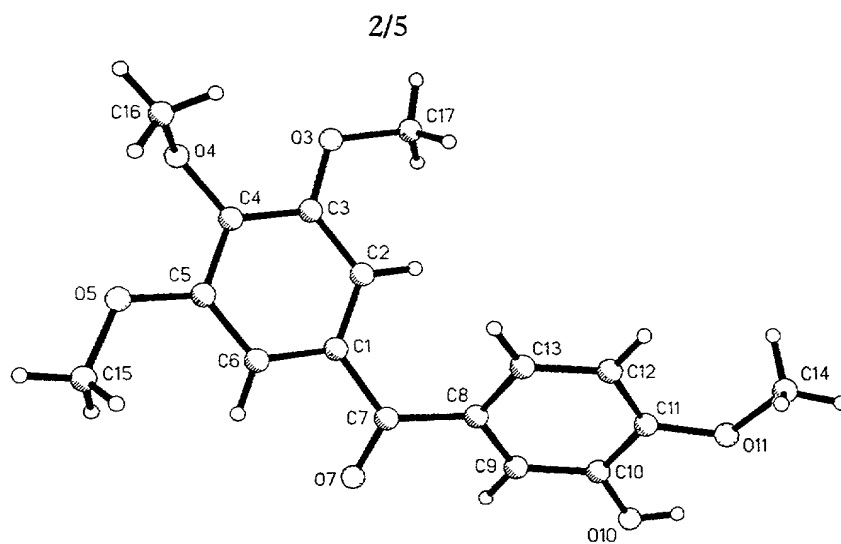
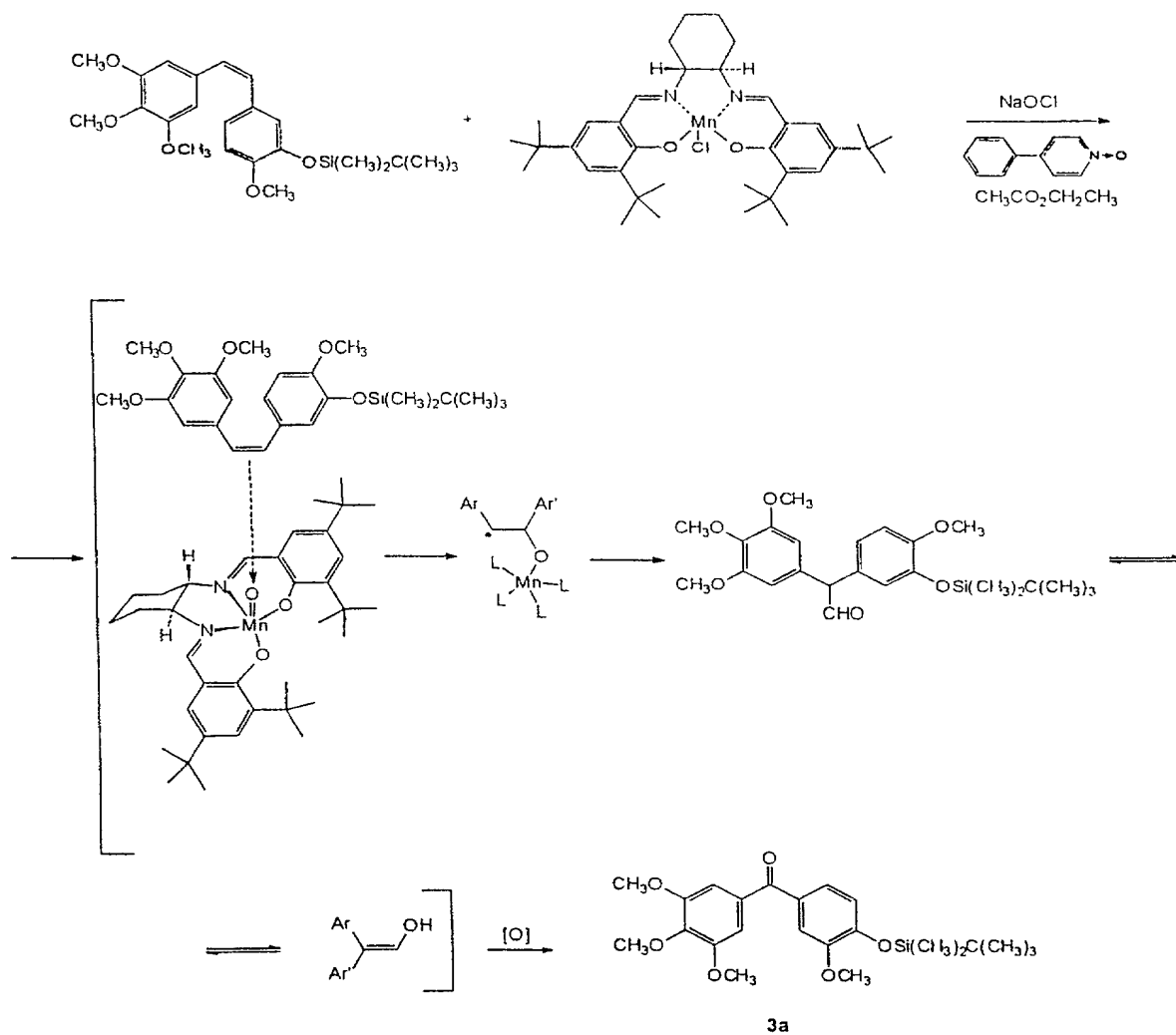


Figure 2.

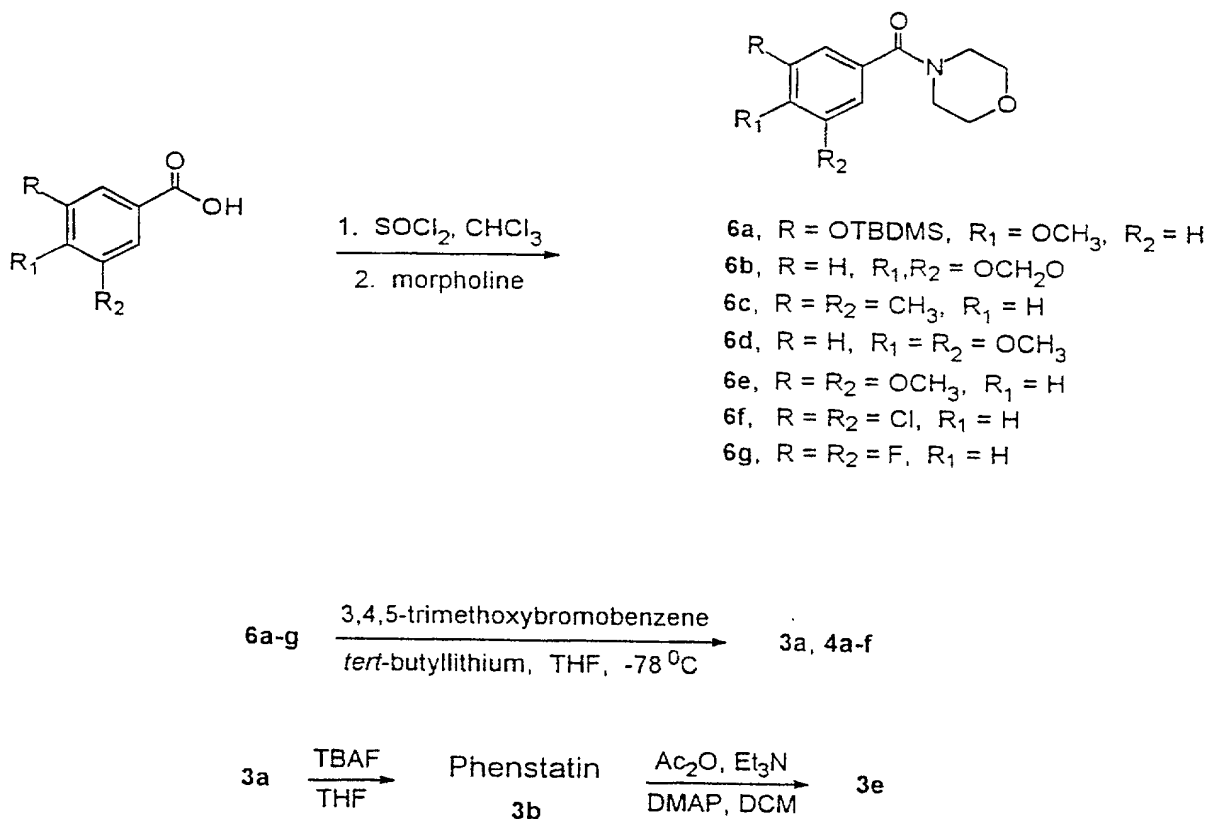
3/5



Scheme 1. Oxidation of Combretastatin A-4 silyl ether (1c) to phenstatin silyl ether (3a).

Figure 3.

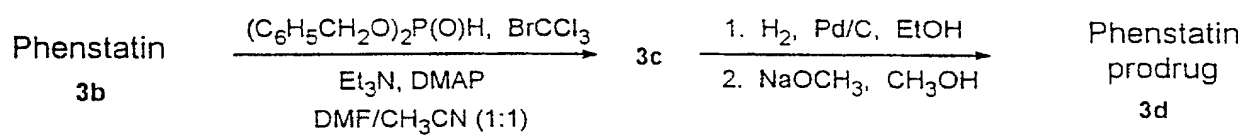
4/5



Scheme 2

Figure 4.

5/5



Scheme 3

Figure 5.

Please type a plus sign (+) inside this box → +

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer
Number Bar Code
Label here

Name	Registration Number	Name	Registration Number
Richard R. Mybeck	17,886		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number OR ☒ Correspondence address below

Name	Richard R. Mybeck				
Address	8010 East Morgan Trail, Suite 10				
Address					
City	Scottsdale	State	AZ	ZIP	85258
Country	US	Telephone	480.483.1285	Fax	480.483.7452

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))	Family Name or Surname
GEORGE R.	PETTIT

Inventor's Signature	<i>George R. Pettit</i>			Date	6/26/00		
Residence: City	Paradise Valley	State	AZ	Country	United States	Citizenship	US
Post Office Address	6232 Bret Hills Drive						
Post Office Address							
City	Paradise Valley	State	Arizona	ZIP	85253	Country	US

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

#3

Please type a plus sign (+) inside this box → ☐

PTO/SB/01 (12-97)

Approved for use through 9/30/00. OMB 0651-0032

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	5367-US
	First Named Inventor	G.R. PETTT
	COMPLETE IF KNOWN	
	Application Number	/
	Filing Date	
	Group Art Unit	
<input type="checkbox"/> Declaration Submitted with Initial Filing	OR	<input type="checkbox"/> Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)
Examiner Name		

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYNTHESIS OF PHENSTATIN AND PRODRUGS THEREOF

the specification of which (Title of the Invention)

☐ is attached hereto
OR☒ was filed on (MM/DD/YYYY) 01/09/1999 as United States Application Number or PCT International

Application Number PCT/US99/00475 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.
60/070,878	01/09/1998	

[Page 1 of 2]

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Please type a plus sign (+) inside this box → ☐

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

BRIAN

TOKI

Inventor's
Signature

Date

7/5/00

Residence: City

LINWOOD

State

WA

Country

United States

Citizenship

US

Post Office Address

16720 6th Avenue, West #C204

Post Office Address

City

Linwood

State

WA

ZIP

98037

Country

United States

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

Inventor's
Signature

Date

Residence: City

State

Country

Citizenship

Post Office Address

Post Office Address

City

State

ZIP

Country

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

Inventor's
Signature

Date

Residence: City

State

Country

Citizenship

Post Office Address

Post Office Address

City

State

ZIP

Country

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.